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The fungal community of wheat phyllosphere was affected by the co-occurrence of stripe rust and powdery mildew

Yan Chen[1], Jiaojiao Jiang[1], Shixian Pu[1,2], Na Wang[3], Ruiming Lin[4], Chengyun Li[1], Lin Liu[1,3]**

[1]*State Key Laboratory for Conservation and Utilization of Bio-resources in Yunnan, Yunnan Agricultural University, College of Plant Protection, Yunnan Agricultural University, Kunming, PR China*
[2]*Zhaotong Company of Yunnan Tobacco Company, Zhaoyang, PR China*
[3]*College of Tobacco Science, Yunnan Agricultural University, Kunming, PR China*
[4]*Institute of Plant Protection, Chinese Academy of Agricultural Science, Beijing, PR China*

**Corresponding authors: licheng_yun@163.com; liulin6032@163.com*

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Abstract: Both wheat stripe rust and powdery mildew are important diseases in the world, which mainly infect the leaves and cause serious yield loss. In this study, the leaf samples of two varieties were collected from different pathogenic processes of stripe rust and powdery mildew that co-occurred in plants, and the internal transcribed spacer (ITS1) amplicon sequencing was introduced to analyse the structure and diversity of phyllosphere fungal communities. The results showed that the alpha diversity indices of the fungal communities were decreased with the pathogenic process, and the beta diversity among the different pathogenic process were significantly different as well. In addition, an Adonis analysis showed the pathogenic processes affected the structure of the fungal community, which could explain 45.6% of the variance in the community structure, on the contrary, the variety has no effect both on the community diversity and the structure. With the development of the pathogenic process, the abundance of both pathogens (*Puccinia striiformis* and *Blumeria graminis*) increased significantly, as well as for the relative abundance of some fungi (i.e., *Alternaria* spp., *Cladosporium* spp., etc.). The relative abundance of other genera (e.g., *Aureobasidium*, *Epicoccum*, etc.) increased at the early pathogenic stage, then decreased at the late pathogenic stage. Comprehensively, these fungi may have the potential to compete with pathogens for nutrients, which may be the target for the development of biological control agents.

Keywords: phyllosphere microbiota; *Puccinia striiformis*; *Blumeria graminis*; diversity; pathogenesis process

The above ground parts of plant, such as the surface and tissues of flowers, fruits, stems and leaves, are generally referred to as the phyllosphere (Lindow & Brandl 2003). Different types of microorganisms (e.g., bacteria, filamentous fungi, yeast and algae) grow, reproduce or temporarily stay in the phyllosphere, which are important components of ecosystem with key ecological functions (Kembel & Mueller 2014; Gao et al. 2016). The relationship between phyllospheric microorganisms and the host plants is very complex, some of which have beneficial effects on the nitrogen fixation, growth promotion and decomposition of re-

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sidual pesticides, while others are pathogens causing diseases and yield loss (Gao et al. 2016). The genetic characteristics of the host plants (Kembel & Mueller 2014; Thapa et al. 2017), the geographical location (Thapa et al. 2018), the seasonal variation and other natural factors, as well as fertiliser (Thapa et al. 2018) and/or pesticide applications (Gu et al. 2010; Karlsson et al. 2017a; Knorr et al. 2019) have a certain impact on the phyllospheric microorganism community.

Compared with studies on rhizosphere microorganisms (Niu et al. 2017; Walters et al. 2018), there are only a few studies on phyllospheric microorganisms. The earliest studies on phyllospheric microorganisms mainly combined artificial cultures and morphological identification, focusing on culturable species or pathogens, as well as their interaction with host plants (Pandey et al. 1993; Król & Machowicz-Stefaniak 2008; Mwashasha et al. 2013; Hantsch et al. 2014). With the development of molecular biology and sequencing technologies, the study of phyllospheric microbiota has been greatly promoted. Most studies have combined artificial cultures and molecular identification, for example, a study on the influence of Camellia grey spot [caused by *Pestalotiopsis maculans* (Cda.) Nag Raj] on fungal communities (Zhang et al. 2011); the diversity of endophytic fungi in sugarcane leaves (Zhou et al. 2017); assessing the impact of an enostroburin application on the bacterial community in the wheat phyllosphere (Gu et al. 2010); Hantsch et al. (2014) studied the genetic correlation between the diversity pattern of epiphytic bacteria in maize leaves and fungal infection resistance. Xu et al. (2014) reported on an analysis of the diversity of endophytes and phyllospheric microorganisms from Yunnan tobacco leaves.

However, 80% of fungi in nature cannot be cultured artificially at present. Therefore, the whole picture of the microbial community cannot be revealed based on culture techniques alone. In recent years, the rapid development of high-throughput sequencing and metagenomics have provided a new perspective for studying the composition and structure of phyllospheric microbiota and their interactions with host plants. Related research studies have gradually emerged, such as a study on endophytic fungi on the leaves of *Metrosideros polymorpha* on the island of Hawaii (Zimmerman & Vitousek 2002), the *Quercus macrocarpa* interfoliar fungus community diversity (Jumpponen & Jones 2009), the phyllospheric fungal community of 51 species in the Panama rainforest (Kembel & Mueller 2014) and a study on the resistance of leaf surface microorganisms of *Arabidopsis thaliana* to grey mould disease (Ritpitakphong et al. 2016). Based on metagenomic technologies, the phyllospheric fungal community and the influence of agronomic measures on the fungal community structure have also been gradually revealed, such as studies on the endophytic fungi within the foliar tissues of *Camellia oleifera* (Zhou et al. 2013), the diversity of endophytic fungi in the leaves of *Dendrobium candidum* (Chen et al. 2015) and tobacco leaf surface microbial diversity (Su et al. 2017); organic farming increases the richness of fungal taxa in the wheat phyllosphere (Karlsson et al. 2017b), and the effect of the fungicide treatment on wheat phyllospheric fungi communities (Karlsson et al. 2017a; Knorr et al. 2019) and so on.

Phyllospheric microorganisms play an important role in agricultural ecosystems. The interaction between them can affect the growth and yield of crops (Luo et al. 2017). However, there are few research studies on the interaction among pathogen-host plant-phyllospheric microbial communities and the influence of a single pathogen on the microbial community. Luo et al. (2017) and Zhang et al. (2017) reported that there were significant differences in the phyllospheric bacterial (Luo et al. 2017) and fungal (Zhang et al. 2018) community structures on pumpkins with different severities of powdery mildew. Pumpkin powdery mildew can change the phyllospheric bacterial and fungal community structures and affect the community diversity. Manching et al. (2014) studied the effect of Southern leaf blight disease [caused by the fungus *Cochliobolus heterostrophus* (Drechs.) Drechs] severity on the phyllosphere's bacterial diversity of maize leaf, the results showed that the disease severity significantly reduced the bacterial species richness, and the application of a nitrogen fertiliser would increase the decline of the bacterial α-diversity. Wang et al. (2016) studied a rhizosphere soil fungal community in a field with different levels of tuber mustard clubroot disease (caused by *Plasmodiophora brassicae*) which showed that the percentage of pathogenic fungi in the heavy tuber mustard clubroot disease field was higher than in other fields, and the tuber mustard clubroot could affect the rhizosphere soil fungal community to some extent.

Wheat is one of the most important crops, and the phyllospheric fungal community of wheat was one of the earliest one's studied (Last 1995). Wheat stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) and wheat powdery mildew (caused by *Blumeria*

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graminis f. sp. *tritici*) are important fungal diseases in the world, and their co-occurrence in the field is a common phenomenon (Dong et al. 1989). Their spores can be spread far away with upper air flow, which has the characteristics of an outbreak and an epidemic in a large area, and leads to serious yield losses or even failure of the harvest. At present, research on the disease cycle and epidemiology of the above diseases are quite clear (Dong et al. 1989; Li et al. 2015; Yan 2016; Ma 2018). However, changes in the community structure of phyllospheric fungi in the pathogenic processes are not clear.

It has been reported that a wheat phyllospheric fungal community is mainly composed of basidiomycete yeasts, saprophytic ascomycetes and plant pathogens (Karlsson et al. 2017a). Different doses, application times and treatments of fungicides have effects on the fungal community of the wheat phyllosphere (Knorr et al. 2019). Sapkota et al. (2015) researched the diversity of wheat, barley, oats and rye, which showed that the host genotype is an important determinant of the cereal phyllosphere microbiota, while the fungicide treatment and geographical location had little effect on the community structure. Karlsson et al. (2017a) studied 22 wheat samples from organically and conventionally cultivated fields in Sweden, and found that the organically cultivated samples significantly increased the species richness of wheat leaves. However, a study on how the wheat pathogens affect the phyllosphere fungal community has not been reported upon.

At present, there are few reports on the high-throughput sequencing technology to study the interactions of the pathogen-crop-phyllosphere microbia in an agricultural ecosystem. In order to further explore the interaction between the pathogens and other microorganisms, we evaluated the diversity and community structure of a phyllospheric fungal community during the co-occurrence of wheat stripe rust and powdery mildew, in order to reveal the mechanism of the wheat disease resistance from the perspective of the microbial ecology, and provide a theoretical basis for the formulation of ecological control strategies.

MATERIAL AND METHODS

Cultivation of wheat plants

Fifteen wheat seed grains of two varieties (being susceptible to wheat powdery mildew) which were identified hosts of *Puccinia striiformis*, Trigo Eureka (T.E., with resistance gene 6) and Abbondanza (A.B., without resistance gene 6) (Wan et al. 2013) were sown in pots (44 cm at the upper diameter and 29 cm high) containing sterilised soil, and placed in a greenhouse on the 12th floor of the engineering centre of Yunnan Agricultural University, which were then moved to a wheat field when the seedlings had grown two leaves. The seedling pots were placed at three points of the wheat field. Each variety at each point was placed in three basins, and the three points were distributed in an equilateral triangle.

Sample collection and processing

The wheat leaves were sampled on the day of placement in the field, which was defined as the pre-infection stage (March 9, 2018), the early pathogenic stage (228 h after placement in the field on March 18, at this time, the initial infection occurred, chlorotic spots appeared on the leaves, and some areas were scattered with a white mould layer, the severity was about 5%) and the late pathogenic stage (444 h after placement in the field on March 27, about 40% of the leaf area was covered by a grey mould layer, and a large number of narrow and long oval uredinium appeared on the leaf tip to the middle of the leaf, and the severity reached 50%). Three representative leaves of each variety and each pot were collected at each sample point, and the collected 10 leaves were placed in an ice box and immediately brought back to the laboratory. Half of the leaves were extracted DNA and the other half were saved as a storage sample.

Sample DNA extraction and amplification sequencing

The DNA of the sampled leaves were extracted according to the instructions of Omega Bio-Tek's E.Z.N.A.® HP fungal DNA Kit (Omega Bio-Tek, Inc., USA), and the DNA samples were purified and tested by polymerase chain reaction (PCR), and then sent to Novogene Biotechnology Co., Ltd, China. For amplification and sequencing. The sequencing region was internal transcribed spacer 1 (ITS1), the upstream primer was ITS5-1737f (5'-GGAAGTAAAAGTCGTAACAAGG-3'), and the downstream primer was ITS2-2043r (5'-GCTGCGTTCTTCATCGATGC-3').

The library was constructed with an Ion Plus Fragment Library Kit 48 rxns kit (Thermo Fisher Scientific, Inc., USA). After qubit quantification

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and qualification, the library was sequenced on the computer with Ion S5^{TM}X.

Data analysis

Cutadapt (Martin 2011) (version 1.9.1, http://cutadapt.readthedocs.io/en/stable/) was used to cut the low-quality reads for the offline data, and then each sample data point was separated from the obtained reads according to a barcode. The raw data were obtained by cutting off the barcode and primer sequence, and then the final effective data were obtained by removing the chimeric sequence. The qualified data were clustered by Uparse software (Edgar 2013), and the sequences were clustered into operational taxonomic units (OTUs) with 97% consistency. At the same time, the representative sequences of the OTUs were selected. The species annotation analysis was carried out by the blast method and with the Unit (version 7.2) database in Qiime software (Caporaso et al. 2010) (version 1.9.1), and the community composition of each sample was counted at each classification level (Kingdom, Phylum, Class, Order, Family, Genus, Species).

The alfa diversity indices of Observed-outs, Chao1, Shannon, Simpson, ACE, etc. were calculated by using Qiime. The dilution curve, rank independence curve, species accumulation curve, analysis of the β-diversity index group differences and the principal coordinate analysis (PCoA) diagram were drawn by using R software (version 2.15.3).

The statistical analysis was conducted by SPSS 16.0. A *t*-test or one-way analysis of variance (ANOVA) was used to do the analysis, the mean value and standard error were used to express the test results, and Duncan's new multiple pole difference method was used to test the significance of the differences between the different treatments in the one-way ANOVA, *P*-value less than 0.05 was considered to have significant differences.

RESULTS

Composition and structure of the wheat phyllospheric fungal community

The total number of raw sequences based on the ITS1 amplification was 2 813 994, and the total number of qualified sequences was 2 809 283. The average number of sequences in each sample was 78 036 ± 6 976. According to the 97% identity cut-off and OTU division, 419 OTUs were obtained, 144 of which were wheat OTUs which were removed from the analysis, the other 267 OTUs were annotated to five phyla of fungi, which included Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota and Zygomycota. The number of OTUs annotated to each phylum was 181, 67, 2, 2 and 15, respectively. A total of 19 classes, 39 orders, 61 families, 63 genera and 132 species were identified (Table 1).

In the wheat phyllospheric fungal community, there was an absolute predominance of Ascomycota, with an average relative abundance of 92.4%. While the average relative abundance of Basidiomycota, Zygomycota, Chytridiomycota and Glomeromycota were 6.9%, 0.4%, 0.1% and 0.2%, respectively. The dominant classes of Ascomycota were Leotiomycetes (87.3%), Dothideomycetes (1.9%), Sordariomycetes (1.0%) and Eurotiomycetes (0.7%); the dominant classes of Basidiomycota were Pucciniomycetes (5.4%), Tremellomycetes (0.7%), Agaricomycetes (0.4%) and Microbotryomycetes (0.2%). Chytridiomycota only include Monoblepharidomycetes and Chytridiomycetes. Glomeromycota only contains Glomeromycetes; Zygomycota only includes incertae sedis Zygomycota (Figure 1A).

Table 1. Sequence summary statistics based on the internal transcribed spacer 1 amplicon sequencing

Items	Count
Total number of raw sequences	2 813 994
Total number of filtered sequences	2 809 283
Mean filtered sequences/sample	78 036 ± 6 976
Number of OTUs	419
OTUs number of wheat	144
OTUs number of fungi	267
Number of OTUs were annotation to each taxon/ No. of taxonomic category	
Phylum	267 OTUs/5 phyla
Ascomycota	181 OTUs
Basidiomycota	67 OTUs
Chytridiomycota	2 OTUs
Glomeromycota	2 OTUs
Zygomycota	15 OTUs
Class	245 OTUs/19 classes
Order	226 OTUs/39 orders
Family	208 OTUs/61 families
Genus	132 OTUs/63 genera
Species	132 OTUs/78 species

OTU – operational taxonomic unit

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There were 132 OTUs annotated to the species level of 78 fungi, the most of which were plant pathogens, with a total of 29 species. Among them, eight species were able to cause wheat diseases, which were *Blumeria graminis*, *Puccinia striiformis*, *Alternaria tenuissima*, *Fusarium oxysporum*, *Magnaporthe oryzae*, *Pseudopithomyces chartarum*, *Monographella cucumerina* and *Parastagonospora nodorum*. The second most abundant ones were saprophytic fungi and yeasts. The saprophytic fungi mainly included a total of 12 species, such as *Aspergillus versicolor*, *Aureobasidium pullulans*, *Cadophora fastigiata*, *Dokmaia monthadangii*, *Mortierella alpina*, *Talaromyces flavus*, *Thelebolus microsporus* and so on. The yeasts mainly included *Cryptococcus diffluens*, *C. rajasthanensis*, *C. uniguttulatus*, *Hannaella oryzae*, *Issatchenkia orientalis*, *Udeniomyces pannonicus*, *Rhodotorula glutinis*, *R. graminis*, *R. mucilaginosa* and *R. terpenoidalis*.

There are 153 common OTUs in all the samples, of which 32 OTUs were annotated to the species level of ten fungi, including *Alternaria tenuissima*, *Aureobasidium pullulans*, *Blumeria graminis*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Microidium phyllanthi* and *Pyrenula paraminarum* from Ascomycota, *Puccinia striiformis* and *Schizophyllum commune* from Basidiomycota, and *Gigaspora margarita* from Glomeromycota. The average relative abundances of *Blumeria graminis* and *Puccinia striiformis* were 20.91% and 1.12%, respectively, and the abundances increased with the development of the disease; the relative abundances of the other eight fungal pathogens of wheat were lower than 0.1%.

Effect of the pathogenic process on the diversity and structure of the fungal community of the wheat phyllosphere

Pathogenic processes affect the α-diversity of the wheat phyllospheric fungal community. Based on the one-way ANOVA of the α-diversity indices (including the Species richness, Shannon, Simpson, Chao1, ACE and PD whole tree) among the different pathogenic processes, the results indicated there was no significant difference between the pre-infection (9th of March) and the early pathogenic stage (18th of March), but the indices of the late pathogenic stage (27th of March), such as the Species richness, Shannon, Simpson and ACE, were

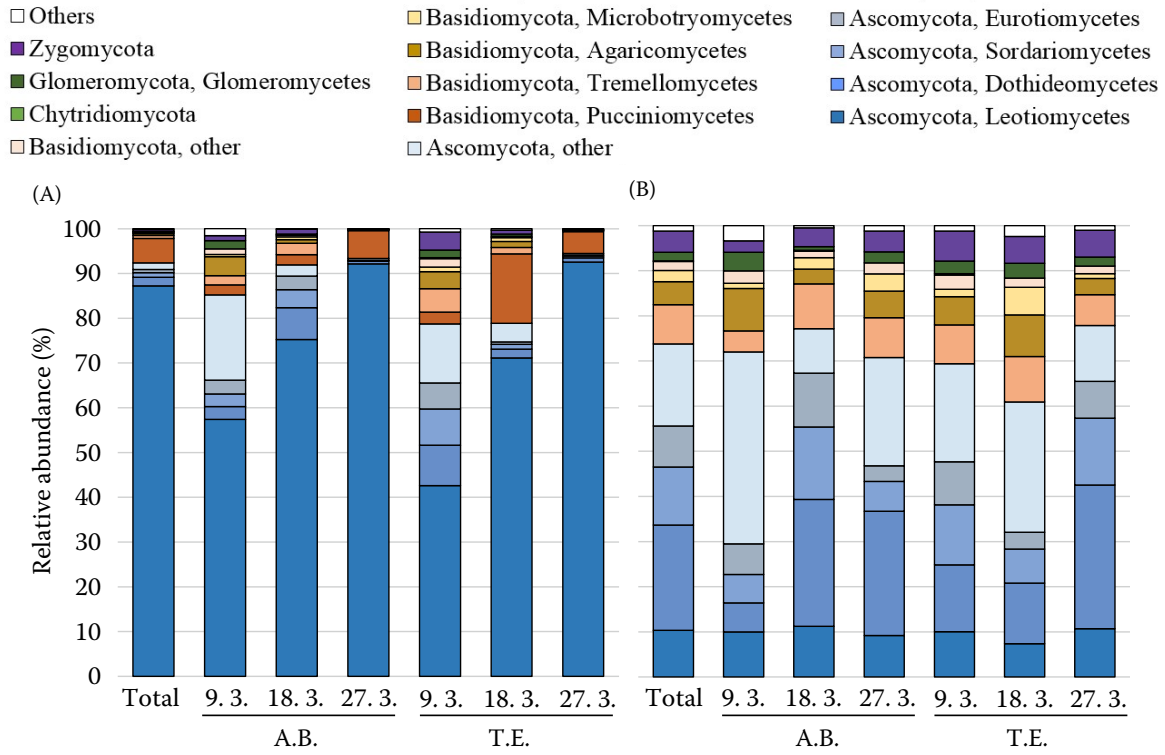

Figure 1. Dynamics of the fungal community composition of the phyllosphere with the pathogenic process. (A) total fungal community composition of the wheat phyllosphere, (B) fungal community composition of the wheat phyllosphere, with *Blumeria graminis* and *Puccinia striiformis* excluded

A.B. – Abbondanza; T.E. – Trigo Eureka

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Table 2. One-way ANOVA results of the α-diversity indices of the phyllosphere fungi communities with different pathogenic processes

Temporal	Richness	Shannon	Simpson	Chao1	ACE	PD whole tree
March 9	58.75 ± 2.40[a]	4.32 ± 0.14[a]	0.90 ± 0.01[a]	90.92 ± 6.10[ab]	74.57 ± 2.89[a]	10.91 ± 0.69[a]
March 18	56.00 ± 4.15[a]	3.91 ± 0.21[a]	0.87 ± 0.01[ab]	111.63 ± 17.04[a]	86.06 ± 6.67[a]	9.70 ± 0.98[a]
March 27	36.42 ± 2.48[b]	3.33 ± 0.14[b]	0.84 ± 0.01[b]	62.11 ± 5.51[b]	50.72 ± 5.34[b]	4.74 ± 0.42[b]

[a]Significant difference at 0.05 level; [b]significant difference at 0.01 level

significantly lower than those in the pre-infection and early pathogenic stage (Table 2).

The result of the paired-samples *t*-test of the α-diversity indices between two varieties showed that there was no significant difference. The species richness of A.B. and T.E. were 51.44 ± 4.00 and 49.33 ± 2.84 (t = 0.677, P = 0.508); the Shannon index was 3.85 ± 0.18 and 3.86 ± 0.15 (t = 0.07, P = 0.945), Chao1 was 100.71 ± 12.59 and 75.73 ± 5.09 (t = 1.94, P = 0.069), ACE was 74.21 ± 6.63 and 66.69 ± 3.87 (t = 1.403, P = 0.179).

Pathogenic processes affect the β-diversity of the wheat phyllospheric fungal community. The β-diversity of the wheat phyllospheric fungal community in the different disease processes was calculated based on the Bray-Curtis distance algorithm, which showed that there were significant differences among the different disease processes, among which the difference between the early stage and the non-disease was 20.65 (P = 0.028); the difference between the late stage and pre-infection stage was 50.17 (P < 0.000 1); the difference between the late pathogenic stage and the early pathogenic stage was –29.52 (P < 0.002) (Figure 2). However, there was no significant difference in the β-diversity between the two varieties (P = 0.336).

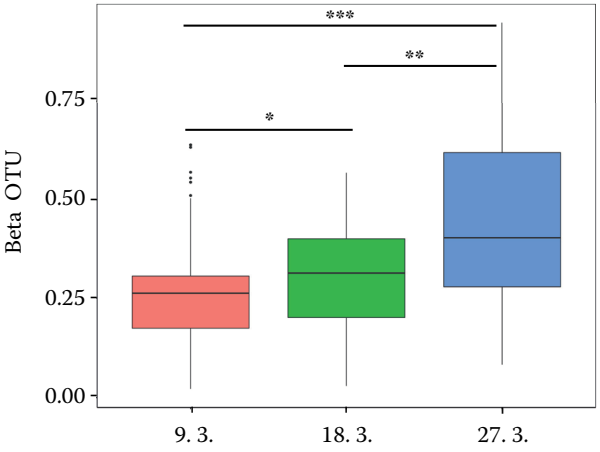

Figure 2. Beta diversity of the phyllosphere fungi communities with different pathogenic processes
OTU – operational taxonomic unit
*P < 0.05; **P < 0.01; ***P < 0.001

The effect of the pathogenic processes on the community structure of the wheat phyllospheric fungi. The course of the disease affects the structure of the wheat phyllospheric fungi community. The Adonis analysis based on the Bray-Curtis algorithm showed that there was no significant difference in the community structure between the early pathogenic stage and the pre-infection stage, but the community structure between the late pathogenic stage and the early pathogenic stage was significant (R^2 = 0.456, P < 0.01), and the community structure between the late stage and the early stage was significantly different (R^2 = 0.345, P < 0.01) (Table 3). The principal coordinate analysis also showed that the samples in the different pathogenic stages was obviously clustering (Figure 3A), however, there was no significant difference in the structure of the phyllospheric fungal community between the two wheat varieties (Figure 3B).

Dynamics of the dominant fungi with the development of the pathogenic processes

Dynamics of the dominant pathogenic fungi in the wheat phyllosphere. Blumeria graminis and *Puccinia striiformis* were the dominant pathogens, and their relative abundance increased gradually with the development of the disease. The relative abundance of *B. graminis* increased from 1.18% to 9.92% and then to 51.64%; the relative abundance of *P. striiformis* increased from 0.05%

Table 3. Adonis test of the category effect on the fungal community by Bray-Curtis distance algorithm

Factor	Compared groups	R^2 (residual)	P-value
Temporal	9. 3.~18. 3.	0.061 (0.939)	0.19
	9. 3.~27. 3.	0.456 (0.542)	0.001
	18. 3.~27. 3.	0.345 (0.655)	0.001
Variety		0.013 (0.987)	0.773

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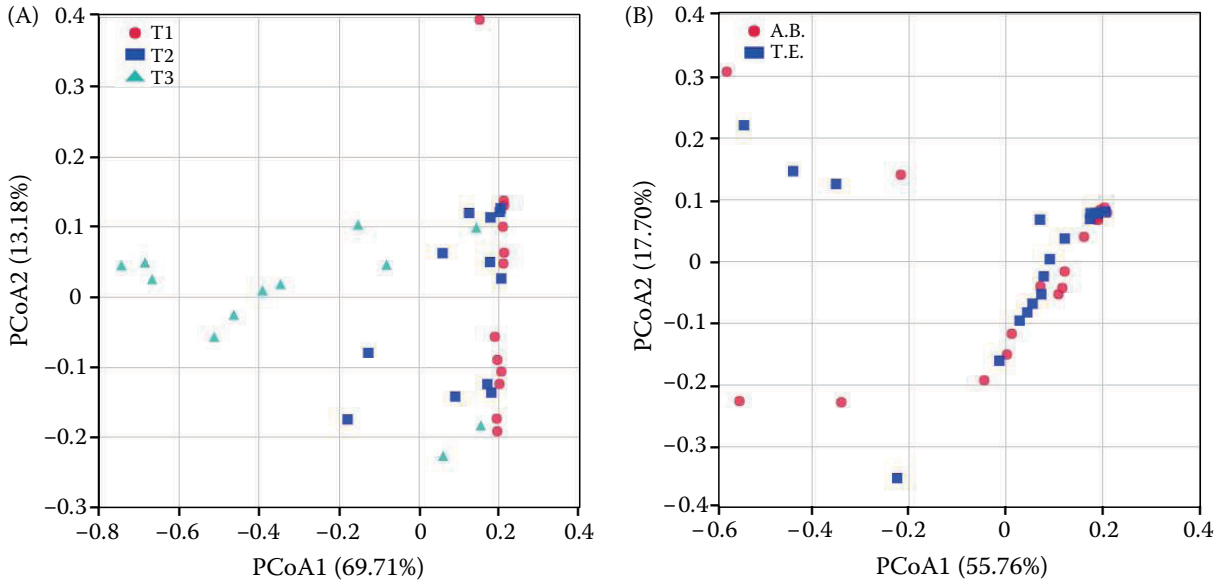

Figure 3. Bray-Curtis matrices visualised using principal coordinates analysis (PCoA) showing (A) the distribution of the samples according to the pathogenic processes in the dataset of the phyllosphere fungal communities of wheat and (B) the distribution of the samples according to the varieties

A.B. – Abbondanza; T.E. – Trigo Eureka; T1 – pre-infection; T2 – early pathogenic; T3 – late pathogenic

to 0.65% then to 2.66%. The relative abundance of *P. striiformis* was significantly higher in the late pathogenesis than that in the pre-infection stage, but there was no significant difference both between the early stage and the pre-infection stage, and between the early pathogenic and late pathogenic stage. The trend of relative abundance of *B. graminis* in variety A.B. was consistent with that of *P. striiformis*. However, the relative abundance of *B. graminis* in variety T.E. was significantly higher in the late stage than in the early pathogenic stage and pre-infection stage, while there was no significant difference in the early stage and pre-infection stage (Figure 4).

The effect of Blumeria graminis and *Puccinia striiformis on the structure of the wheat phyllospheric fungal community.* In the whole stage of mixed occurrence of wheat stripe rust and powdery mildew, after removing the proportion of wheat powdery mildew and stripe rust, Ascomycota and Basidiomycota were mainly detected in the wheat phyllosphere, and their relative abundance gradually decreased with the disease process. The relative abundance of Zygomycota and Glomeromycota also decreased with the progress of the disease; Chytridiomycota was only present in a few samples (Table 4).

However, with the development of the pathogenic process, the order of the dominant class changed in variety A.B., Leotiomycetes and Eurotiomycetes were the dominant class of Ascomycota at the pre-infection stage, while Dothideomycetes became dominant with the development of the pathogenesis; Agaricomycetes and Tremellomycetes were dominant in Basidiomycota, while the relative abundance of Tremellomycetes became higher than that of the Agaricomycetes. In the T.E. variety, the dominant class of Ascomycota and Basidiomycota was unchanged with the development of the pathogenic process (Figure 1B).

The dynamics of the pathogenic and non-pathogenic fungi of wheat with the pathogenic process. The OTU number (Figure 5A) and relative abundance (Figure 5C) of the wheat fungal pathogens in the early and late pathogenic stages were significantly higher than that in the pre-infection stage, although the species number (Figure 5B) of the wheat pathogenic fungi in the three stages had no significant difference in both the A.B. and T.E. varieties.

While for the non-wheat pathogenic fungi, there was definitely a difference between the two varieties, but there was no significant difference in OTU number, species number and relative abundance among the three periods in the T.E. variety, but the OTU number, species number and relative abundance in the early pathogenic stage were significantly higher than that of pre-infection stage and late pathogenic stage (Figures 5D, 5E, 5F).

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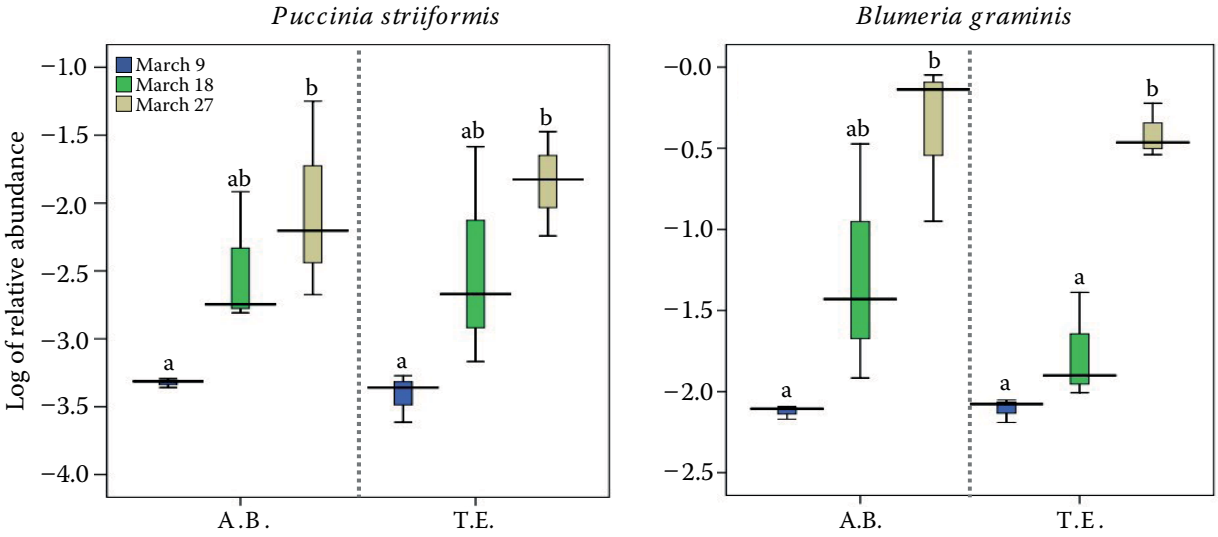

Figure 4. The relative abundance of *Puccinia striiformis* and *Blumeria graminis* both increased significantly with the pathogenic process

One-way ANOVA and Duncan's multiple range test at $P < 0.05$ were used to analyse the data within the different pathogenic processes

A.B. – Abbondanza; T.E. – Trigo Eureka

[a,b]Indicate a significant difference among the different treatments

The OTU number, species number and relative abundance of the two varieties in the different sampling periods were analysed via the paired *t*-test. The results showed that the OTU number and species number of the wheat pathogens and non-pathogens had no significant difference between the two varieties, but the relative abundance of the non-pathogens of the A.B. variety was significantly higher than that of the T.E. variety. In the early pathogenesis stage, the relative abundance of the non-wheat pathogens of the A.B. variety was significantly higher than that of the T.E. variety, but there was no significant difference between the two varieties in the other two periods (Figure 5).

Table 4. Relative proportion (%) of the dominant class with the pathogenic process

Dominant class	Abbondanza			Trigo Eureka		
	March 9	March 18	March 27	March 9	March 18	March 27
Ascomycota						
Leotiomycetes	4.48	2.84	0.17	6.09	1.06	0.31
Dothideomycetes	2.88	7.11	0.52	9.04	1.94	0.92
Sordariomycetes	2.83	4.05	0.12	8.08	1.08	0.43
Eurotiomycetes	3.04	3.02	0.07	5.82	0.54	0.24
Other	19.09	2.50	0.45	13.21	4.16	0.36
Basidiomycota						
Tremellomycetes	2.08	2.51	0.17	5.24	1.46	0.20
Agaricomycetes	4.22	0.83	0.11	3.82	1.33	0.10
Microbotryomycetes	0.51	0.64	0.07	0.99	0.88	0.03
Other	1.23	0.36	0.05	1.90	0.29	0.05
Chytridiomycota	0.00	0.06	0.00	0.21	0.00	0.00
Glomeromycota	1.86	0.20	0.05	1.70	0.47	0.06
Zygomycota	1.13	1.05	0.09	4.04	0.86	0.17
Others	1.52	0.13	0.02	0.73	0.35	0.03

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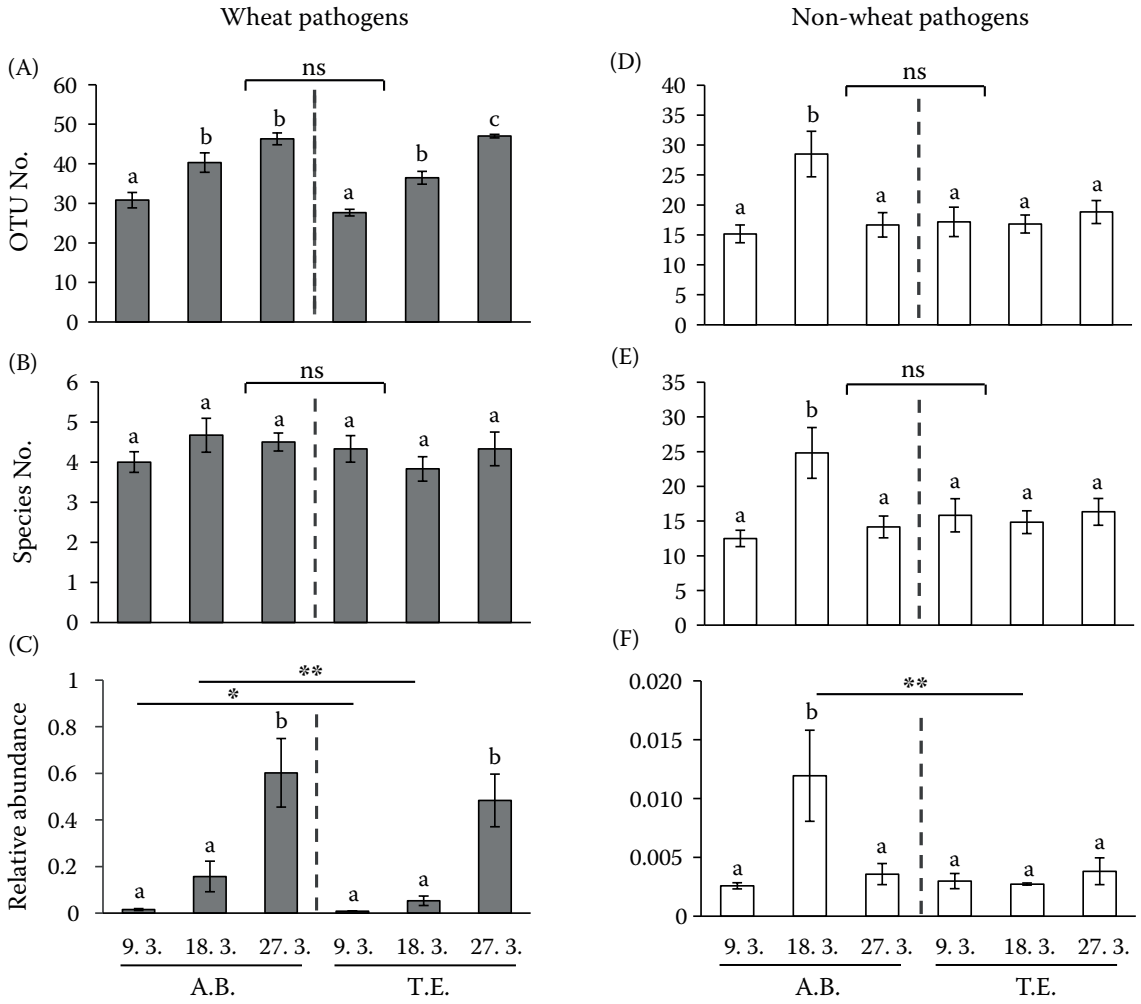

Figure 5. Changes in the operational taxonomic unit (OTU) number, species number and relative abundance of the pathogenic and non-pathogenic fungi in the wheat at different stages

A.B. – Abbondanza; T.E. – Trigo Eureka

**Indicate the significance level of the *F*-values ($P < 0.01$)

[a,b]Indicate a significant difference among the different treatments

One-way ANOVA and Duncan's multiple range test at $P < 0.05$ were used to analyse the data within the different pathogenic process, values are presented as the mean ± SE; *t*-test was used to analyse the data within the different varieties

Fungi species with abundance changes significantly with the pathogenic process. The results of the hypothesis testing the relative abundance at the genus level among the different disease stages via the MetaSta method indicated the relative abundance of *Aureobasidium*, *Epilococum*, *Issatchenkia*, *Rhodotorula*, *Sporobolomyces* and *Udeniomyces* increased in the early stages of the pathogenesis and decreased in the late pathogenesis (Figure 6). In addition, the abundance of the saprophytic fungi or necrotrophic pathogens, such as *Alternaria* spp., *Cladosporium* spp. and *Leptosphaeria* sp., increased with the pathogenic progression.

DISCUSSION

Diversity of fungal community on wheat phyllosphere

In general, the dominant species annotated in this study are consistent with previous reports which used high-throughput sequencing (Blixt et al. 2009; Sapkota et al. 2015; Karlsson et al. 2017a; Su et al. 2017) and traditional culture methods (Larran et al. 2007; Miao 2011), and mainly included *Alternaria* spp., *Cladosporium* spp., *Fusarium oxysporum* and *Rhizopus arrhizus* of Ascomycota, *Sporobolomyces roseus*, *Cryptococcus*

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spp. and *Udeniomyces* spp. of Basidiomycota, besides *Blumeria graminis* and *Puccinia striiformis*.

In this study, Ascomycota and Basidiomycota were the dominant phylum of the wheat phyllospheric fungal community, which was consistent with Sapkota's research about fungicides affecting the structure of the wheat phyllospheric fungal community (Sapkota et al. 2015), whose result showed that the wheat phyllospheric fungal community was mainly comprised of basidiomycete yeasts, saprophytic ascomycetes and plant pathogens. It was also consistent with the results of Zhang et al. (2018) on the pumpkin powdery mildew disease caused by *Spharotheca cucurbiae* (Jacz.) Z. Y. Zhao, whose severity influences the fungal diversity of the phyllosphere (Zhang et al. 2018).

The effect of the pathogenic process on the fungal community of the wheat phyllosphere

At present, there is no report on the effect of mixed occurrence of wheat stripe rust and powdery mildew on the fungal diversity and structure of the phyllosphere. In this study, the result showed that the α-diversity indices (Species richness, Chao1, ACE) of the fungal community decreased with the pathogenic process, which is consistent with the research about "Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness" (Manching et al. 2014). While, in the report on pumpkin powdery mildew disease, the severity influences the fungal diversity of the phyllosphere (Zhang et al. 2018), which indicated that the pathogenic fungi infection may promote an increase in the richness at the initial stage, and then decrease with an increase in the disease severity due to the biotic pressure (i.e., symbiotic and competitive stresses among the microbial species). Taken together, it is hypothesised that different pathogens may have different effects on the microbial community structure of the host phyllosphere, thus it is necessary to focus on this hypothesis in future research.

The T.E. and A.B. varieties are both susceptible to *Blumeria graminis* and *Puccinia striiformis* infections, so the diversity of the fungal community decreases with an increase in the disease pressure. The co-occurrence of wheat stripe rust and powdery mildew can change the structure of the fungal community of phyllosphere based on present study. With an increase in the disease level, *B. graminis* and *P. striiformis* gradually become dominant, while the abundance of other fungi changes with the dis-

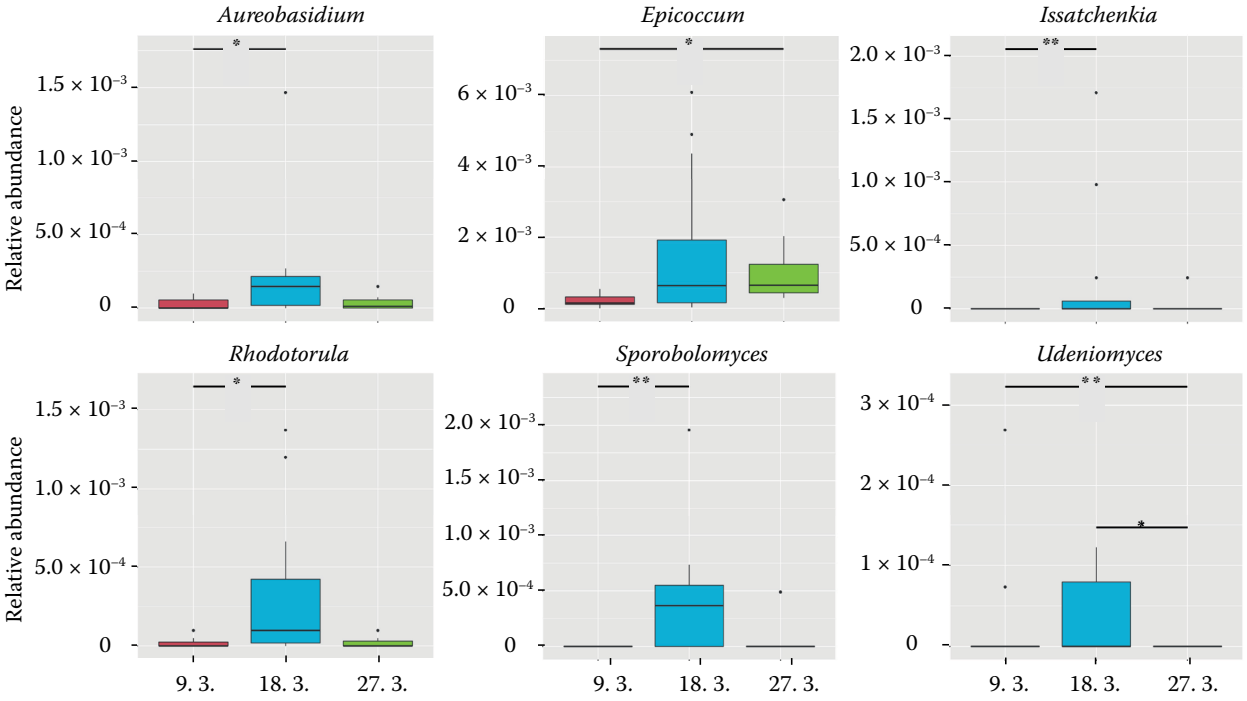

Figure 6. The genera of the phyllosphere fungi with the relative abundance increased at the early pathogenesis stage then decreased at the late pathogenesis stage

*$P < 0.05$; **$P < 0.01$

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ease process, which is consistent with the research results that powdery mildew can change the structure of bacterial (Luo et al. 2017) and fungal communities (Zhang et al. 2018) of the pumpkin phyllosphere, and was also consistent with the research of Zhang et al. (2011) on the effect of *Camellia* grey spot on the foliar fungal community.

Currently, there are few reports about the interaction among the pathogen-plant-microbial community of the phyllosphere, while both the infection of *Blumeria graminis* and *Puccinia striiformis* is mainly initiated through the leaves, therefore to study the influence of those pathogens on the wheat foliar fungal community can provide reference for revealing their disease resistance mechanism and formulating ecological and biological control strategies from the perspective of microbial ecology.

The effect of the variety on the community structure of phyllospheric fungi

Previous reports indicate that the variety may have an influence on the diversity and structure of phyllospheric fungal communities, for example, Liu et al. (2008) used the traditional culture method to study the fungi flora in wheat leaf with different resistance to wheat leaf blight, which indicated that there was little difference in the leaf epiphytic fungal flora between resistant varieties and susceptible varieties, but the composition of the endophytic fungal flora was significantly different, and the endophytic fungal flora of the susceptible varieties was more than that of the resistant varieties. Wang et al. (2012) also showed that there was a negative correlation between the number of fungi and the resistance of flue-cured tobacco varieties. The difference in the disease resistance can be reflected by the difference in the microbial species, quantity, distribution and diversity, but it may also be different due to the difference in the crops and diseases. Sapkota et al. (2015) showed that the host genotype at the species and variety level played an important role in the formation of the foliar fungal community. However, there were no significant differences in the α-diversity, β-diversity and community structure between the two wheat varieties in the present study mainly because of no obviously resistant difference to the stripe rust and powdery mildew for the A.B. and T.E. varieties, both of them were susceptible to these diseases. Additionally, the results may have limitations since only two wheat varieties were used in this study, several varieties with significant resistance differences can be used for future research.

Potential biological control agents against wheat powdery mildew and stripe rust

Currently, the control of wheat powdery mildew and stripe rust is mainly based on the combination of chemical controls and the cultivation of resistant varieties. However, due to the rapid variation in the physiological races of *Blumeria graminis* and *Puccinia striiformis*, it is easy for the varieties to lose resistance, in addition to the impact of chemical pesticides on the environment and the rapid emergence of pathogens' resistance (Gong 2007; Ma 2018), it is urgent to develop more new control measures. Therefore, the use of microorganisms to control diseases has become a new research direction. There have been some reports on the biological control of wheat powdery mildew (Fan 2005; Fan 2009; Cao 2017), but few related reports exist on wheat stripe rust.

Although the biomass of the microorganisms on the surface and in the tissues of plants seems to be negligible, the existing studies suggest that the microorganisms can exert influence on the plants through their own metabolites or by means of signal transduction (He 2005). Their existence may promote the adaptation of plants to the environment and strengthen the ecological balance of the system. Therefore, utilising original habitat microorganisms in micro-ecosystems for plant disease control is conducive to reducing the selection pressure on the pathogens, as well as being a good strategy for resistance management because they can co-evolve with the pathogens (Lu 2010). At present, there are many reports about endophytic microorganisms to control plant diseases, which have played a great role in the biological control of bacterial diseases, fungal diseases and nematodes (Lin 2008).

There are a large number of yeasts on the surface of plants, and because of their high safety, rapid growth and reproduction, strong adaptability to the environment and little influence by chemical agents, they can also be used together with chemical agents, which are widely used in the prevention and control of postharvest diseases of fruits and vegetables (Zhang et al. 2003). For example, *Candida saitoana* (Ahmed et al. 2003), which was used to control the postharvest decay of apple fruit;

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Cryptococcus infirmo-miniatus and *Rhodotorula glutinis* (Benbow & Sugar 1999), which were applied to control the postharvest diseases of pears; *Pichia caribbica*, which was applied to control the postharvest rot of strawberries (Jin et al. 2013), *Candida intermedia*, which was applied to control onion black mould (Ling & Chi 2002), and so on.

Epicoccum nigrum, which is widespread in the air, soil and plant tissues, can rapidly produce conidia on the surface of dying plant tissues, and produce flavipin, epicorazines A and B and other antibacterial substances to inhibit spore germination, can also produce enzymes to change the cell membrane permeability of pathogens and degrade the cell wall. It is an ideal biological control agent (BCA) for applications to control crop diseases (Li et al. 2010). Researchers from Canada and Spain have used their spore suspension to control pea sclerotinia rot (Ting & Reeleder 1989) and peach twig blight (Madrigal et al. 1994) in the field with good control efficacy. In addition, studies have shown that *E. nigrum* have an antagonistic effect on the pathogens of *Diplodia pinea* [causes shoot blight of *Pinus sylvestris* var. *mongolica* (Li et al. 2004)] and *Cochliobolus sativus* (Campbell 1956). In addition, Zhang (2014) found that the endophytic fungi of rape such as *Alternaria* spp., *Aureobasidium* spp. and *Epicoccum* spp. can produce indoleacetic acid, which can inhibit the growth of *Sclerotinia* spp.

In this study, we found that the abundance of genera, such as *Aureobasidium*, *Epicoccum*, *Issatchenkia*, *Rhodotorula*, *Sporobolomyces* and *Udeniomyces*, increased in the early pathogenic stage, and decreased in the late pathogenic stage, which indicated that they probably have nutritional competition with *Blumeria graminis* and *Puccinia striiformis* in the early stage of infection. The abundance of *Alternaria* spp., *Cladosporium* spp., and *Leptosphaeria* sp. gradually increased with the development of the disease, which may indicate that they also have nutritional competition with the two pathogens, may have potential antagonistic effect, and have the potential to develop BCAs for these two diseases. However, on the one hand, the research may be limited (i.e., only two wheat varieties used in the experiment) and on the other hand, the relationship between the pathogen-plant-microbial community is very complex (Kembel & Mueller 2014; Gao et al. 2016), which needs more research to verify the hypothesis.

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REFERENCES

Ahmed E.G., Wilson C.L., Wisniewski M. (2003): Control of postharvest decay of apple fruit with *Candida saitoana* and induction of defense responses. Phytopathology, 93: 344–348.

Benbow J.M., Sugar D. (1999): Fruit surface colonization and biological control of postharvest diseases of pear by preharvest yeast applications. Plant Disease, 83: 839–844.

Blixt E., Olson Å., Lindahl B., Djurle A., Yuen J. (2009): Spatiotemporal variation in the fungal community associated with wheat leaves showing symptoms similar to stagonospora nodorum blotch. European Journal of Plant Pathology, 126: 373–386.

Campbell W.P. (1956): The influence of associated microorganisms on the pathogenicity of *Helminthosporium sativum*. Canadian Journal of Botany, 34: 865–874.

Cao Y.Y., Wang W.L., Shen L.L., Xu X.F., Li T.Y. (2017): Screening of bacterioides TMG-8 for bio-control of wheat powdery mildew. Jiangsu Agricultural Sciences, 45: 95–99.

Caporaso J.G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F.D., Costello E.K., Fierer N., Peña A.G., Goodrich J.K., Gordon J.I., Huttley G.A., Kelley S.T., Knights D., Koenig J.E., Ley R.E., Lozupone C.A., McDonald D., Muegge B.D., Pirrung M., Reeder J., Sevinsky J.R., Turnbaugh P.J., Walters W.A., Widmann J., Yatsunenko T., Zaneveld J., Knight R. (2010): QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7: 335–336.

Chen Z.B., Li B., Wang D.K., Yu L., Liu J.N., Xu S.G., Jin S., Ren Z. (2015): Diversity of endophytic fungi in leaves of *Dendrobium candidum*. Fujian Journal of Agricultural Science, 30: 978–983.

Dong J.Z., Zeng S.M., Ma Q.X. (1989): Epidemic processes of wheat yellow rust, leaf rust and powdery mildew when they occur together in the field. Scientia Agricultura Sinica, 22: 67–71.

Edgar R.C. (2013): UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nature Methods, 10: 996–998.

Fan Y.G., Cao Y.Y., Wei S.H., Wang M.L. (2005): Screening and curbing efficacy of potential biocontrol agents for powdery mildew of wheat. Journal of Shenyang Agricultural University, 36: 541–545.

https://doi.org/10.17221/107/2021-PPS

Fan Y.G., Wang L., Dong H.Q., Cao Y.Y. (2009): Inhibiting effects of bio-control agents of wheat powdery mildew on different pathogenic fungi. Journal of Henan Agricultural Sciences, 38: 92–97.

Gao S., Liu X.C., Dong Z., Liu M.Y., Dai L.Y. (2016): Advance of phyllosphere microorganisms and their interaction with the outside environment. Plant Science Journal, 34: 654–661.

Gong Y.F. (2007): Biological control of powdery mildew of wheat by endophytic bacterial [MSc. thesis]. Yangling, Northwest Agriculture and Forestry University.

Gu L.K., Bai Z.H., Jin B., Hu Q., Wang H.L., Zhuang G.Q., Zhang H.X. (2010): Assessing the impact of fungicide enostroburin application on bacterial community in wheat phyllosphere. Journal of Environmental Sciences, 22: 134–141.

Hantsch L., Braun U., Haase J., Purschke O., Scherer-Lorenzen M., Bruelheide H. (2014): No plant functional diversity effects on foliar fungal pathogens in experimental tree communities. Fungal Diversity, 66: 139–151.

He M.X. (2005): Research status and progress on fungal endophyte as a biocontrol factor. Plant Protection, 31: 10–14.

Jin S.S., Pang S.X., Zhao L.N., Zhang H.Y., Zhang X.Y., Shu Z.L. (2013): Screening and identification of an antagonistic yeast and its application for biological control of postharvest decay of strawberries. Food Science, 34: 202–208.

Jumpponen A., Jones K.L. (2009): Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate *Quercus macrocarpa* phyllosphere. New Phytolosist, 184: 438–448.

Karlsson I., Friberg H., Steinberg C., Persson P. (2017a): Fungicide effects on fungal community composition in the wheat phyllosphere. PLoS One, 9: e111786. doi: 10.1371/journal.pone.0111786

Karlsson I., Friberg H., Kolseth A.K., Steinberg C., Persson P. (2017b): Organic farming increases richness of fungal taxa in the wheat phyllosphere. Molecular Ecology, 26: 3424–3436.

Kembel S.W., Mueller R.C. (2014): Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. Botany, 92: 303–311.

Knorr K., Jørgensen L.N., Nicolaisen M. (2019): Fungicides have complex effects on the wheat phyllosphere mycobiome. PLoS One, 14: e0213176. doi: 10.1371/journal.pone.0213176

Król E., Machowicz-Stefaniak Z. (2008): Biotic effect of fungal communities inhabiting grapevine phyllosphere on *Phoma negriana*. Biologia, 63: 466–470.

Larran S., Perelló A., Simón M.R., Moreno V. (2007): The endophytic fungi from wheat (*Triticum aestivum* L.). World Journal of Microbiology and Biotechnology, 23: 565–572.

Last F.T. (1995): Seasonal incidence of *Sporobolomyces* on cereal leaves. Transactions of the British Mycological Society, 38: 221–239.

Li B.N., Xu S.H., Yang C.B., Huang J.L., Pang L.J., Shi T.S., Liu X.G., Li Y.M., Xiang C.T., Xie Y.H. (2004): Screening of antagonistic fungus for *Pinus sylvestris* var. *mongolica* shoot blight. Journal of Northeast Forestry University, 32: 97–99.

Li Y., Wang Y.N., Hu T.L., Cao K.Q. (2010): Progress of research and application of *Epicoccum purpurascens* Ehreube × Schlecht (= *E. nigrum* Link) in biological control of plant diseases. Journal of Anhui Agricultural Sciences, 38: 2988–2990.

Li Y., Gu Y.L., Wu B.M., Jin S.L., Cao S.Q., Wang X.M., Sun Z.Y., Luo Y., Ma Z.H. (2015): Establishment of a duplex TaqMan real-time PCR method for quantifying *Puccinia striiformis* f. sp. *tritic*i and *Blumeria graminis* f. sp. *tritici*. Acta Phytopathologica Sinica, 45: 205–210.

Lin L., Qiao Y.S., Gu B.K., Zhou Y.J., Dong H.S. (2008): Advances in the study of plant endophytic bacteria and their effects on biological control of plant disease. Jiangsu Journal of Agricultural Sciences, 24: 969–974.

Lindow S.E., Brandl M.T. (2003): Microbiology of the phyllosphere. Applied and Environmental Microbiology, 69: 1875–1883.

Ling Q.F., Chi Z.F. (2002): Study on antagonistic effect and mechanism by *Candida intermedia* for biological control of moulds on vegetables and fruits. Food and Fermentation Industries, 28: 34–38.

Liu S.F., Zhang M., Li H.L. (2008): Study on the fungi flora in wheat leaf with different resistance to wheat leaf blight. Journal of Henan Agricultural University, 42: 216–219.

Lu X.F., Luo K., Xie B.Y., Chen G.H., Mao Z.C. (2010): Metagenomics and its application in biological control of plant diseases. Chinese Journal of Biological Control, 26: 106–112.

Luo L.Y., Zhang Z., Jin D.C., Tan X.Q., Zhang D.Y., Tian Y., Lu X.Y., Liu Y. (2017): Phyllosphere bacterial diversities and community structures on pumkin with different severities of powdery mildew. Acta Phytopathologica Sinica, 47: 688–695.

Ma Z.H. (2018): Researches and control of wheat stripe rust in China. Journal of Plant Protection, 45: 1–6.

Madrigal C., Pascual S., Melgarejo P. (1994): Biological control of peach twig blight (*Monilinia laxa*) with *Epicoccum nigrum*. Plant Pathology, 43: 554–561.

Manching H.C., Balint-Kurti P.J., Stapleton A.E. (2014): Southern leaf blight disease severity is correlated with decreased maize leaf epiphytic bacterial species richness and the phyllosphere bacterial diversity decline is enhanced by nitrogen fertilization. Frontiers in Plant Science, 5: 403. doi: 10.3389/fpls.2014.00403

Martin M.J. (2011): Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal, 17: 10–12.

https://doi.org/10.17221/107/2021-PPS

Miao W.L. (2011): Diversity of endophytic fungi in wheat (*Triticum aestivum* L.) and their relationship with the host [MSc. thesis]. Zhengzhou, Zhengzhou University.

Mwajita M.R., Murage H., Tani A., Kahangi E.M. (2013): Evaluation of rhizosphere, rhizoplane and phyllosphere bacteria and fungi isolated from rice in Kenya for plant growth promoters. SpringerPlus, 2: 606. doi: 10.1186/2193-1801-2-606

Niu B., Paulson J.N., Zheng X., Kolter R. (2017): Simplified and representative bacterial community of maize roots. Proceedings of the National Academy of Sciences, 114: E2450–E2459.

Pandey R.R., Arora D.K., Dubey R.C. (1993): Antagonistic interactions between fungal pathogens and phylloplane fungi of guava. Mycopathologia, 124: 31–39.

Ritpitakphong U., Falquet L., Vimoltust A., Berger A., Métraux J.P., L'Haridon F. (2016): The microbiome of the leaf surface of *Arabidopsis* protects against a fungal pathogen. New Phytologist, 210: 1033–1043.

Sapkota R., Knorr K., Jorgensen L.N., O'Hanlon K.A., Nicolaisen M. (2015): Host genotype is an important determinant of the cereal phyllosphere mycobiome. New Phytologist, 207: 1134–1144.

Su J.K., Xu D., Guo L., Luo J.M., Shao D.Y., Yun F., Cai J.B. (2017): Diversity analysis of microorganism on flue-cured tobacco leaf surface based on metagenome sequencing. Genomics and Applied Biology, 36: 1538–1545.

Thapa S., Prasanna R., Ranjan K., Velmourougane K., Tamakrishnan B. (2017): Nutrients and host attributes modulate the abundance and functional traits of phyllosphere microbiome in rice. Microbiological Research, 204: 55–64.

Thapa S., Ranjan K., Ramakrishnan B.K., Velmourougane K., Prasanna R. (2018): Influence of fertilizers and rice cultivation methods on the abundance and diversity of phyllosphere microbiome. Journal of Basic Microbiology, 58: 172–186.

Ting Z., Reeleder R.D. (1989): Application of epicoccum-purpurascens spores to control white mold of snap bean. Plant Disease, 73: 639–642.

Walters W.A., Zhao J., Nicholas Y., Wallace J.G., Sutter J., Zhang W., González-Peña A., Peiffer J., Koren O., Shi Q.J., Knight R., Glavina D.R.T., Tringe S.G., Buckler E.S., Dangl J.L., Ley R.E. (2018): Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proceedings of the National Academy of Sciences, 115: 7368–7373.

Wan A.M., Jia Q.Z., Jin S.L., Li G.B., Wang B.T., Yao G., Yang J.X., Yuan Z.Y., Bi Y.Q. (2013): Pathogenic changes of stripe rust fungus of wheat in China during 1997–2001. Acta Phytopathological Sinica, 33: 261–266.

Wang G., Yang H.W., Zhao Z.X., Li F.L., Yi J.H. (2012): Microbe quantity and functional diversity in rhizospheres of different cultivars of flue-cured tobacco. Plant Nutrition and Fertilizer Science, 18: 451–458.

Wang D.D., Tian X.L., Pan L.M. (2016): Study on rhizosphere soil fungal community in field with different levels of tuber mustard clubroot disease. Northern Horticulture, 13: 115–118.

Xu H., Yang G.H., Zhang M., Dang L.Z., Wang W.Y., Wang M.F., Duan Y.Q. (2014): Analysis of diversity of endophytes and phyllospheric microorganism from Yunnan tobacco leaves. Journal of Yunnan Agricultural University, 29: 149–154.

Yan Z.Y. (2016): Remote sensing of wheat powdery mildew and stripe rust and spore concentration dynamics of *Blumeria graminis* f. sp. *triciti* in the field [MSc. thesis]. Beijing, Chinese Academy of Agricultural Science.

Zhang Q.H. (2014): Evaluation of the diversity and biocontrol potential of the endophytic fungi in oilseed rape [PhD. thesis]. Wuhan, Huazhong Agricultural University.

Zhang H.Y., Jiang Y.H., Zheng X.D., Xi W.Y.F., Sun P. (2003): Research advances on the biological control of postharvest diseases of fruits and vegetables with yeast. Transactions of the Chinese Society of Agricultural Engineering, 19: 23–27.

Zhang L.N., Zhu T.H., Yang Z.Z., Zhang J.P. (2011): Influence of Camellia gray spot disease on foliar fungal communities. Journal of Sichuan Agricultural University, 29: 378–385.

Zhang Z., Luo L.Y., Tan X.Q., Kong X., Yang J.G., Wang D.H., Zhang D.Y., Jin D., Liu Y. (2018): Pumpkin powdery mildew disease severity influences the fungal diversity of the phyllosphere. PeerJ, 6: e4559. doi: 10.7717/peerj.4559

Zhou S.L., Yan S.Z., Wu Z.Y., Chen S.L. (2013): Detection of endophytic fungi within foliar tissues of *Camellia oleifera* based on rDNA ITS sequences. Mycosystema, 32: 819–830.

Zhou N., Zhao X.L., Xie Q.W., Bai X.F. (2017): Diversity of endophytic fungi in sugarcane leaves. Jiangsu Agricultural Sciences, 45: 93–95.

Zimmerman N.B., Vitousek P.M. (2002): Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proceedings of the National Academy of Sciences, 109: 13022–13027.

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